DNA Sequencing in Arrays of Glass Microchannels

Joseph W. Balch[1], Courtney Davidson[1], Larry Brewer[1], Joe Kimbrough [1],Steve Swierkowski[1], Jackson Koo[1], David Nelson[1], Paula Mc Cready[1], Robert Bruce[1], Ray Mariella[1],and Anthony Carrano[1]. [1]Biology and Biotechnology Program, Lawrence Livermore National Laboratory, Livermore Ca 94550.

We are investigating microchannel arrays on single glass substrates as an alternative technology to arrays of discrete capillaries for DNA sequencing. We have developed an advanced microfabrication process which can be used to etch channels of arbitrary width and depth (e.g. 50-1000 μm wide and 10-200 μm deep) in large area glass plates (typically 7 x 50 cm). Once etched the microchannel structure is completed by thermally bonding a top plate with input and output ports for sample introduction and buffer reservoir interconnects. This technology approach provides a number of advantages for building large arrays of electrophoresis microchannels for DNA sequencing. By fabricating the array of microchannels on a single glass substrate, the arrays of microchannels are very robust mechanically and can be handled without any special care. By means of photolithography and chemical etching techniques the dimensions of rectangular cross-section channels can be optimized by making the channel depth thin to minimize the thermal dispersion of DNA bands while at the same time the channel width can be made large to increase the amount of dye-labeled DNA available for strong fluorescence signal generation and detection. The detection of the fluorescence signal is also made easier by having a flat optical window over the channels through which laser excitation of fluorescence occurs with less scattered light of the primary laser beam to contribute to the overall noise level.

Using these microfabrication technologies we have built various test electrophoresis plates and arrays of microchannels. Channels of various cross section sizes have been built and tested using low viscosity solutions of linear poly(dimethylacrylamide). Recent experimental results of microchannels 60 um deep by 250 um wide and having a 38 cm load-to-read length resulted in greater than 400 base resolution in about 90 min. for a 160 V/cm separation field. Arrays of 12, 24 and 96 microchannels have been built and are being tested. We presently are building and assembling a 96 channel system based on this technology and will report on-going results obtained from it for high throughput DNA sequencing.

Work is supported by a grant from the National Center for Human Genome Research, National Institutes of Health and by The Department of Energy Human Genome Program. Work was performed by Lawrence Livermore National Laboratory under auspices of the U. S. Department of Energy under contract no. W-7405-ENG-48.